[GSTALIVN]-x(2)-H-E-[LIVMFYW]-{DEHRKP}-H-x-[LIVMFYWGSPQ] (SEQ ID NO: 8) (ProSite Accession No. PS00142 and ProSite Documentation PDOC00129; for information regarding ProSite prefixes, refer to Sonhammer et al. (1997) *Protein* 28:405-420). ECE-1 also has a number of cysteine residues that are conserved among, at least one of which is involved in disulphide-linked homodimerization and other(s) that can be palmitoylated.--

C1

Please replace the paragraph bridging pages 12 and 13 with the following:

--A representative human ECE-1 amino acid sequence (*i.e.*, NM 001397) is predicted to have the following: ten putative N-glycosylation sites (PS00001); one putative glycosaminoglycan attachment site (PS00002); three putative cAMP- and cGMP-dependent protein kinase phosyphorylation sites (PS00004); ten putative protein kinase C phosphorylation sites (PS00005); sixteen to eighteen putative casein kinase II phosphorylation sites (PS00006); three putative tyrosine kinase phosphorylation sites (PS00007); nine to ten putative N-myristoylation sites (PS00008); and one putative neutral zinc metallopeptidase zinc-binding region signature domain (PS00142). In addition, glycosylation can be important for full enzymatic activity of ECE-1. The above examples are representative only, and those of skill are aware that any position along an ECE nucleic acid sequence represents a site of potential mutation.--

C2

Please replace the paragraph bridging pages 22 and 23 with the following:

--In particular, the sandwich ELISA used herein was performed as follows: 96-well microtiter plates were coated overnight at 4°C with 100 µl of a 5 µg/ml dilution of primary antibody in sodium carbonate coating buffer (SCCB; 0.1 M Na_2CO_3, pH 9.6). Plates were blocked overnight at 4°C with 300 µl of BLOCKACE Solution (PBS + 1.0% BLOCKACE (Snow Brand Milk Products, Japan), 0.05% NaN_3, pH 7.4). Samples for analysis and synthetic Aβ standards (Bachem, Switzerland) were diluted in buffer EC (0.02 M NaH_2PO_4, 0.002 M EDTA, 0.4 M NaCl, 0.2% BSA, 0.05% CHAPS, 0.04% BLOCKACE, 0.05% NaN_3, pH 7.0) and allowed to incubate on the plates overnight at 4°C. Plates were washed twice with PBS (8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 139 mM NaCl, 2.7 mM KCl, pH 7.4) and 100 µl of secondary

C3

antibody directly coupled to HRP (EZ-LINK™ Plus Activated Peroxidase kit (contents of kit: peroxidase, 5 M sodium cyanoborohydride solution ($NaBH_3CN$), quenching buffer (3 M ethanolamine, pH 9.0), BupH™ phosphate buffered saline, Buph™ carbonate-bicarbonate buffer), according to manufacturers directions; Pierce Chemical Co., Rockford, IL) was allowed to bind either 4 hrs at room temperature or overnight at 4°C. Plates were then washed twice with PBS containing 0.05% Tween 20 followed by two additional washes in PBS. Detection was performed using TMB (3,3',5,5'-tetramethyl-benzidine) as an HRP substrate according to the manufacturer's specifications (Kirkegaard & Perry Laboratories (KPL), Gaithersburg, MD) and the reaction stopped by the addition of 100 µl of 1N H_3PO_4. Plates were read at 450 nm in a SpectraMax Plus spectrophotometer (Molecular Devices; Sunnyvale, CA) and analyzed by SOFTmax® PRO software. Aβ40 or Aβ42 were quantitated by comparison with the values obtained for each synthetic Aβ standard from the same plate.--

C3

Please replace the paragraph on page 25, lines 1-16 with the following:

--Cells were passaged into 6-well plates one day prior to treatment and grown to confluence. Triplicate wells were washed twice with Hank's balanced salt solution and then incubated for 17-24 hrs with 1 ml of growth medium containing phosphoramidon (34-100 µM), thiorphan (Sigma) or captopril (Sigma) at the appropriate concentrations. Control cells were incubated in growth medium containing an equal volume of vehicle (PBS). After treatment, the culture medium was harvested, spun at 14,000 xg, and the supernatant analyzed for Aβ40 or Aβ42 using sandwich ELISA and for secreted APP using Western blot. To assess cellular toxicity of the compounds, MTS assays (CELLTITER 96® (contents: tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS[(a)]] and phenazine ethosulfate (PES)), Promega, Madison, WI), which measure the conversion of MTS to formazan by metabolically active cells, were performed on the cells after the indicated times. Culture medium was subjected to electrophoresis on 10-20% Tricine gels (Novex, Carlsbad, CA) and was subsequently transferred to Immobilon P (Millipore, Bedford, MA). Western blots on CHO cells were performed using a 22C11 antibody (Boehringer) to detect secreted APP. Bound antibody was detected by incubation with the appropriate HRP-

C4